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Patentanmeldung Nr. Patent application No. Demande de brevet n°

03023395.1

Der Präsident des Europäischen Patentamts;
Im Auftrag

For the President of the European Patent Office

Le Président de l'Office européen des brevets
p.o.

R C van Dijk

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Bezeichnung der Erfindung/Title of the invention/Titre de l'invention:
(Falls die Bezeichnung der Erfindung nicht angegeben ist, siehe Beschreibung.
If no title is shown please refer to the description.
Si aucun titre n'est indiqué se referer à la description.)

Stabilized peptides

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Stabilized Peptides

Field of the Invention

A common principle of the structure of many naturally occurring proteins is the presence of helical domains. Usually, such helical parts of proteins contain 20-30 amino acid residues and are a typical element of the secondary structure of proteins. Typical examples of such proteins are cytokines like interleukin 2 (Majewski 1996) , interleukin 4 (Gustchina et al. 1997, 1995) and interleukin 6 (Somers et al. 1997), but others like erythropoietin also contain helical substructures (Sytkowski and Grodberg 1997), which usually participate in the cytokine/receptor interactions. In such proteins helices are frequently assembled around a hydrophobic core, to which hydrophobic amino acid residues project. Around this core they form tertiary structures, for which coiled coil interactions of the type also called "leucine zipper" are typical (Vieth et al. 1994). By this overall constructive principle, such helix-bundle cytokines project their contact surface to the outside of the molecule, while the hydrophobic core forms a stable anchor, around which the helical subdomains cluster. The surface tension of the surrounding aqueous solvent thus is the source of forces, which finally stabilizes the helical parts around the hydrophobic core.

Binding domains of such helix-bundle type cytokines are interesting parts of the molecule and it is tempting to just take the sequence of 20-30 amino acids and to use this part of the molecule alone to bind to the receptor (Theze et al. 1999). This

would aim and would be suitable for both antagonistic or agonistic approaches to minimized cytokines or cytokine antagonists. Moreover, such short stretches can be obtained in a relatively uncomplicated manner by means of classical chemical solid phase peptide synthesis of the Merrifield-type.

Object of the invention

However, reduction to smaller peptides takes away the above mentioned constructive principles and short peptides of 30 amino acids are only partially helical in aqueous solutions (Theze et al. 1999). The hydrophobic side chains, which projected to the former core part of the complete cytokine are now projecting into the aqueous surrounding, which is a destabilizing factor for the helix and induces a tendency of such molecules to aggregate in irregular clusters. Such clusters might have some residual activity, but sometimes this is just due to irregular ligand/receptor interactions, which are different from the natural type of interactions (Eckenberg et al. 2000; Theze et al. 1999). The helical structure, which is needed to bind to the receptor molecules is thus not stable in water. Frequently, the overall helical content, measured by circular dichroism is below 50%. This does not even mean that 50% of the molecules are completely helical, but indicates that the average over all helical interactions is 50%. The actual concentration of completely helical molecules which are needed for adequate binding, is not known and certainly much smaller than 50% of the apparent molar concentration. For all these reasons, the binding constants of small peptides to a given receptor usually do neither qualitatively nor quantitatively match the cytokine

receptor interactions although they might harbour a sequence-identical subpart of the molecule.

For the isolation of helical structures from proteins, e.g. cytokines, one has to solve the problem of stabilisation of helices by means of other methods than assembly around a large hydrophobic core.

Several approaches have been reported for stabilizing alpha-helical peptides. Stabilization can be achieved by addition of trifluorethanol or hexafluorisopropanol (Sung and Wu 1996). A variety of non-covalent side chain constraints have been reported including incorporation of metal chelates, salt bridges, and hydrophobic interactions. However, the non-covalent strategies suffer from a number of disadvantages:

Solvents like trifluoroethanol or hexafluorisopropanol increase helical content, but can not be used in pharmaceutical preparations and are certainly not present in sufficient concentrations in vivo.

Metal chelates and salt bridges induce very large highly polar groups, which – in case of small peptides – are likely to negatively influence the binding to the receptor as well as pharmacokinetic properties of a given molecule. In case of metal chelates only non-toxic metals can be used for pharmaceutically relevant preparations.

Hydrophobic interactions are difficult to control. Irregular aggregation and undesired intermolecular interactions usually form problems, which induce a great loss in active substance being available after a complex synthesis and preparation protocol. Sometimes, only the use of strong detergents and/or organic solvents is able to control aggregation thoroughly. Again, such preparations are difficult to apply to the intended pharmaceutical targets.

Thus, one of the most successful strategies is the stabilization of alpha-helical peptides by covalent bridges which connect the side chains of two appropriately located amino acids. Appropriately implies that the side chains of these amino acids do not participate in the intended binding sites. Moreover, they should stabilize two helical turns, which means a step of 7 amino acids in the sequence. Bridges which connect two sidechains at positions i and i+7 can stabilize the helix with little perturbation on helix conformation. Once the helical conformation is enforced by such a construct, the overall helical content improves strongly and the total helical conformation of the peptide becomes kinetically favoured. The constraints of peptides by lactamization (Huston et al. 1995), amides (Braisted et al. 1998; Braisted et al. 1997) or disulfide bonds (Jackson et al. 1991) have been described. However, all these strategies suffer from the disadvantage, that the synthetic strategy has to be designed such that the closure of the constraint structure of the side chain is possible. In case of disulphide bonds, this becomes difficult as soon as other disulphide bridges have to be closed.

Summary of the invention

The present invention therefore presents modules, from which helical constraints can be built by very flexible strategies. The peptide bonds involved partially compensate the hydrophobic nature of the disulphide bonds, which are also included into the constraint strategy. Thus, the invention presents solutions, by means of which peptide bonds or closure of disulphide bridges can be used alternatively for closure of the constraint. This offers greater synthetic flexibility. Moreover, the peptide bonds are more hydrophilic than disulphide bridges alone and offer the advantage of better solubility of the product in an aqueous surrounding. It is possible to attach solvation tags like glycosyl moieties, polyethylenglycol or other suitable extensions or appendices to the helical constraint structure. Usually, such a hydrophilic helical constraint structure replaces two hydrophobic amino acid side chains and thus improves pharmacologic properties of the molecule.

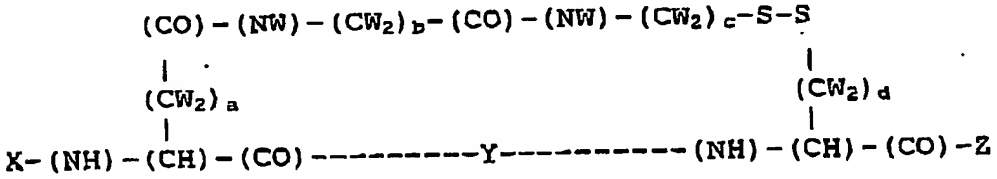
Detailed description of the invention

In general, the invention provides structures which can be adapted to almost every synthetic problem during the synthesis of helically stabilized peptides. Structurally, the bridges, which are constructed alongside the sequence of the peptide, comprise a flexible covalent backbone with at least one amide bond and one disulphide bond. Closure of the bridge by the disulphide bond will e. g. be a good way of formation of the bridge. But if necessary, the bridge can be closed e.g. by

on-resin closure of one peptide bond, while the disulphide bridge was already introduced as a ready to use building block. The skilled person knows other possible ways or is able to find other possible ways for performing the invention after reading and understanding the present description of the invention.

Below, a series of six general formulas will present the whole range of the invention. The invention encompasses helical constrained peptides represented by formula (1) to (7).

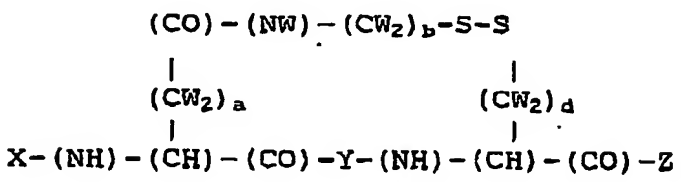
Formula (1) represents a compound



(1)

wherein X is hydrogen or any amino acid or any peptide, Y is any amino acid sequence consisting of six amino acids, Z is hydroxyl or any amino acid or any peptide, a, b, c and d are independently selected from the integers 1 to 3, provided that a+b+c+d is any integer in the range from 5 to 9; at each independent position of W, W can be freely chosen from hydrogen, a hydroxyl-, carboxyl- or amino group, an alkyl moiety with at least one hydroxyl-, carboxyl- or amino group, a polyethyleneglycol moiety, or a naturally occurring or artificial sugar molecule, and the peptides can consist of natural and/or unnatural D- and/or L-amino acids. Examples 1 to 4 demonstrate the application of this formula.

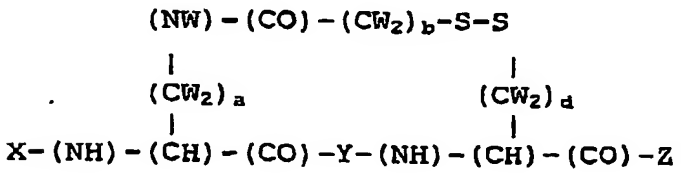
Formula (2) represents a compound



(2)

wherein X is hydrogen or any amino acid or any peptide, Y is any amino acid sequence consisting of six amino acids, Z is hydroxyl or any amino acid or any peptide, a, b and d are independently selected from the integers 1 to 5, provided that a+b+d is any integer in the range from 7 to 11; at each independent position of W, W can be freely chosen from hydrogen, a hydroxyl-, carboxyl- or amino group, an alkyl moiety with at least one hydroxyl-, carboxyl- or amino group, a polyethyleneglycol moiety, or a naturally occurring or artificial sugar molecule, and the peptides can consist of natural and/or unnatural D- and/or L-amino acids. Example 5 illustrates this formula.

Formula (3) represents a compound

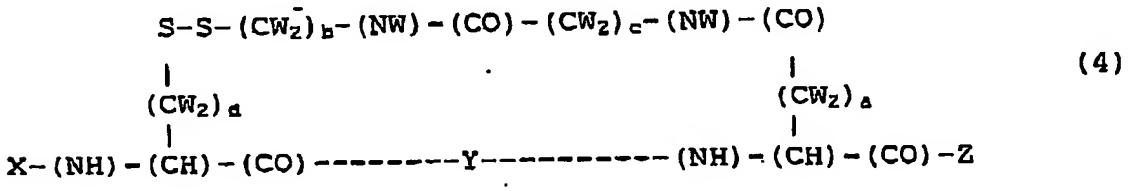


(3)

wherein X is hydrogen or any amino acid or any peptide, Y is any amino acid sequence consisting of six amino acids, Z is hydroxyl or any amino acid or any peptide, a, b and d are independently selected from the integers 1 to 5, provided that a+b+d is any integer in the range from 7 to 11; at each independent position

of W, W can be freely chosen from hydrogen, a hydroxyl-, carboxyl- or amino group, an alkyl moiety with at least one hydroxyl-, carboxyl- or amino group, a polyethyleneglycol moiety, or a naturally occurring or artificial sugar molecule, and the peptides can consist of natural and/or unnatural D- and/or L-amino acids. Example 6 illustrates this formula.

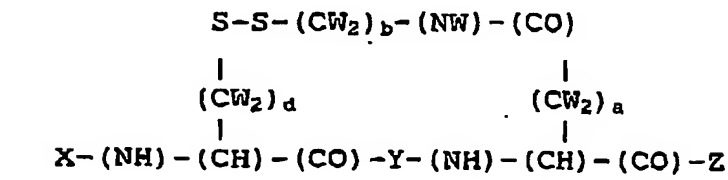
Formula (4) represents a compound



(4)

wherein X is hydrogen or any amino acid or any peptide or any compound represented by formula (1) to (2), Y is any amino acid sequence consisting of six amino acids, Z is hydroxyl or any amino acid or any peptide or any compound represented by formula (1) to (6), a, b, c and d are independently selected from the integers 1 to 3, provided that a+b+c+d is any integer in the range from 5 to 9 and the peptides can consist of natural and/or unnatural D- and/or L-amino acids; at each independent position of W, W can be freely chosen from hydrogen, a hydroxyl-, carboxyl- or amino group, an alkyl moiety with at least one hydroxyl-, carboxyl- or amino group, a polyethyleneglycol moiety, or a naturally occurring or artificial sugar molecule, and the peptides can consist of natural and/or unnatural D- and/or L-amino acids. Example 7 illustrates the application of this formula.

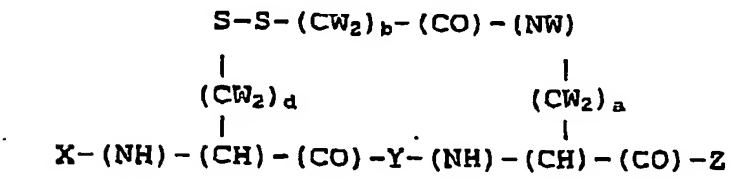
Formula (5) represents a compound



(5)

wherein X is hydrogen or any amino acid or any peptide or any compound represented by formula (1) to (6), Y is any amino acid sequence consisting of six amino acids, Z is hydroxyl or any amino acid or any peptide or any compound represented by formula (1) to (6), a, b and d are independently selected from the integers 1 to 5, provided that a+b+d is any integer in the range from 7 to 11 and the peptides can consist of natural and/or unnatural D- and/or L-amino acids; at each independent position of W, W can be freely chosen from hydrogen, a hydroxyl-, carboxyl- or amino group, an alkyl moiety with at least one hydroxyl-, carboxyl- or amino group, a polyethyleneglycol moiety, or a naturally occurring or artificial sugar molecule, and the peptides can consist of natural and/or unnatural D- and/or L-amino acids. Example 8 illustrates this type of formula.

Formula (6) represents a compound



(6)

wherein X is hydrogen or any amino acid or any peptide or any compound represented by formula (1) to (6), Y is any amino acid sequence consisting of six

amino acids, Z is hydroxyl or any amino acid or any peptide or any compound represented by formula (1) to (6), a, b and d are independently selected from the integers 1 to 5, provided that a+b+d is any integer in the range from 7 to 11 and the peptides can consist of natural and/or unnatural D- and/or L-amino acids; at each independent position of W, W can be freely chosen from hydrogen, a hydroxyl-, carboxyl- or amino group, an alkyl moiety with at least one hydroxyl-, carboxyl- or amino group, a polyethyleneglycol moiety, or a naturally occurring or artificial sugar molecule, and the peptides can consist of natural and/or unnatural D- and/or L-amino acids. Examples 9 and 10 illustrate the application of this formula.

Amino acids described in this invention can be of the naturally occuring L stereoisomer form as well as the enantiomeric D form. The one-letter code refers to the accepted standard polypeptide nomenclature, but can mean alternatively a D- or L-amino acid:

Code	amino acids
A	L-Alanine or D-Alanine
V	L-Valine or D-Valine
L	L-Leucine or D-Leucine
I	L-Isoleucine or D-Isoleucine
M	L-Methionine or D-Methionine
F	L-Phenylalanine or D-Phenylalanine

Y	L-Tyrosine or D-Tyrosine
W	L-Tryptophan or D-Tryptophan
H	L-Histidine or D-Histidine
S	L-Serine or D-Serine
T	L-Threonine or D-Threonine
C	L-Cysteine or D-Cysteine
N	L-Asparagine or D-Asparagine
Q	L-Glutamine or D-Glutamine
D	L-Aspartic acid or D-Aspartic acid
E	L-Glutamic acid or D-Glutamic acid
K	L-Lysine or D-Lysine
R	L-Arginine or D-Arginine
P	L-Proline or D-Proline
G	Glycine

By way of a non-limiting example a constraint building block was prepared as follows.

Cysteamine (10mmol) was dissolved in 20ml trifluoroacetic acid. The solution was stirred at room temperature and a solution of acetamidomethanol (12mmol) was added dropwise over a period of 30 minutes. The mixture was stirred for additional 120 minutes and the volatile parts removed in vacuo. The residue was dissolved in 80ml water and the pH adjusted to 9. The product was then extracted with chloroform/isopropanol (3/1) and the solvents removed in vacuo. The crude

product was then dissolved in a minimum of DCM and this solution added to a mixture of BOC-ß-Ala (10mmol), Cl-HOBt (10mmol), DIEA (10mmol) and DIC (20mmol) in a minimum of DCM. After 12 hours the solution was washed with satured sodiumhydrogencarbonate, sodiumhydrogensulfate and sodiumchloride, dried over sodiumsulfate and the solvent removed in vacuo. The residue was dissolved in 10ml trifluoroacetic acid and stirred for 60 minutes. Trifluoroacetic acid was then removed by coevaporation with DCM and the residue dissolved in DCM. The solution was neutralized by addition of DIEA and poured into a mixture of Fmoc-Glu-OtBu (10mmol), Cl-HOBt (10mmol), DIEA (10mmol) and DIC (20mmol). After 12 hours the solution was washed with satured sodiumhydrogencarbonate, sodiumhydrogensulfate and sodiumchloride, dried over sodiumsulfate and the solvent removed in vacuo. The residue was dissolved in 10ml trifluoroacetic acid and stirred for 60 minutes. Trifluoroacetic acid then was removed by coevaporation with DCM and the residue dissolved in DCM. The crude constraint building block was purified by HPLC on a Kromasil C-18 column, eluted with acetonitrile-water gradient containing 0.1% v/v trifluoroacetic acid and lyophylized.

The sequences given in the examples below harbour target specific sequences, which can be used in the way described below, but might be modified without loss of desired action by means of single or multiple amino acid exchange operations. A substitution mutation of this sort can be made to change an amino acid in the resulting peptide in a non-conservative manner (i.e. by changing an amino acid belonging to a grouping of amino acids having a particular charge or size or other

characterisitics to a grouping of amino acids with other grouping parameters) or in a conservative manner (i.e. by changing amino acids within one grouping of amino acids). Such a conservative change generally leads to less change in the structure and function of the resulting protein. A non-conservative change is more likely to alter the structure, activity or function of the resulting protein, although – if done at the right place – might be without deleterious effect on the target-interactions. The present invention should be considered to include sequences containing conservative and non-conservative changes, which do not significantly alter the activity or binding characteristics of the resulting modified peptide as compared to the original sequence. The following is one example of various groupings of amino acids:

Amino acids with nonpolar R Groups:

Alanine, Valine, Leucine, Isoleucine, Proline, Phenylalanine, Tryptophan, Methionine

Amino acids with uncharged polar R Groups:

Glycine, Threonine, Serine, Cysteine, Tyrosine, Asparagine, Glutamine

Amino Acids with charged polar R groups (negatively charged at pH 6):

Aspartic Acid and Glutamic Acid

Basic Amino Acids (positively charged at pH 6)

Lysine, Arginine, Histidine

Amino Acids with phenyl groups:

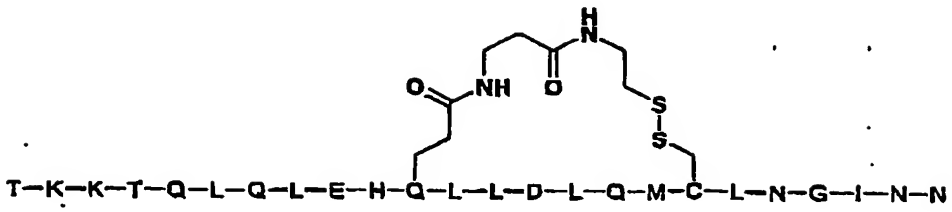
Phenylalanine, Tryptophan, Tyrosine

Particularly preferred conservative substitutions are: Lys for Arg and vice versa; Glu for Asp and vice-versa; Ser for Thr and vice versa; Gln for Asn and vice versa.

Moreover, the invention includes modifications of the given binding regions of the peptide by amino acids, which transfer specific desired properties to the peptide. Such improvements include N- and/ot C-terminal modifications, which protect the peptides against exopeptidas cleavage. Preferred solutions of this problem include the use non-natural amino acids in terminal positions, especially preferred is the use of D-amino acids. Non-conservative exchanges inside the peptide sequence might be used to transfer better water solubility to non-binding but hydrophobic regions of the peptide. Chelating amino acids in N- or C-terminal postions can be use to enable the peptide to bind to metal-activated surfaces in order to assist purification and refolding during the production process.

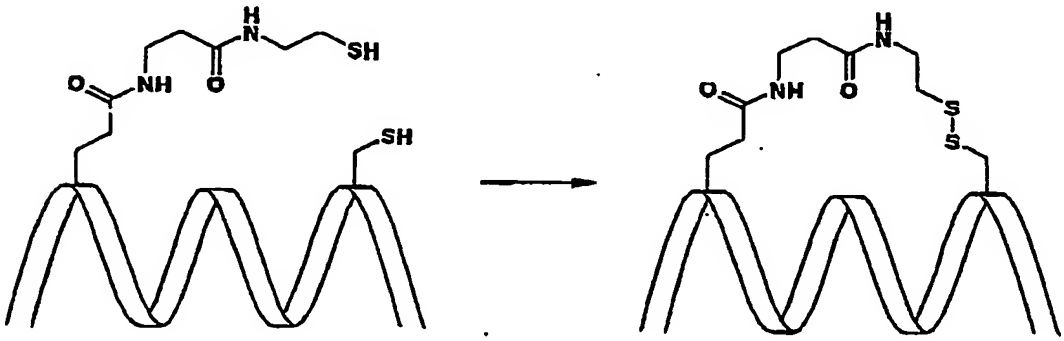
Examples

Example 1:

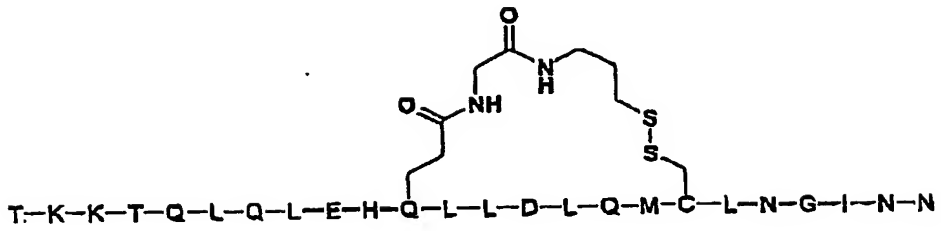


The bridge in example 1 connects the side chains of glutamine (glutamic acid respectively) and cysteine via beta-alanine and 2-aminoethanthiol. This compound represents an antagonist for the interleukin-2 receptor.

The last step of the synthesis of the cyclic helical constraint bridge is normally the formation of a disulfide bridge:

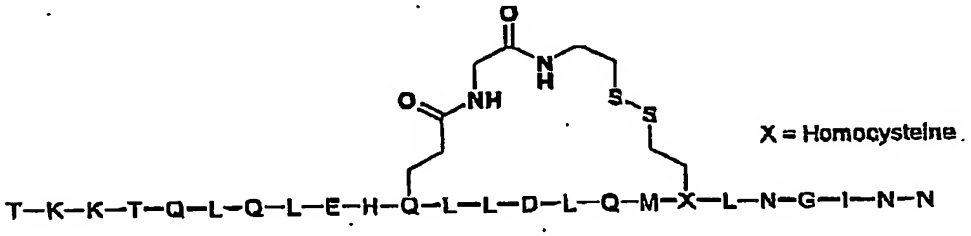


Example 2:



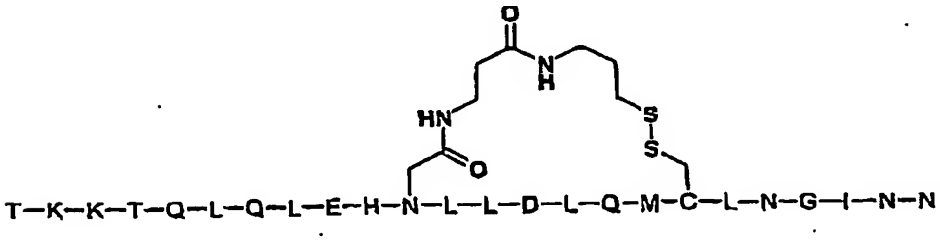
The bridge in example 2 connects the side chains of glutamine (glutamic acid respectively) and cysteine via glycine and 3-aminopropan-1-thiol. This compound represents an antagonist for the interleukin-2 receptor.

Example 3:



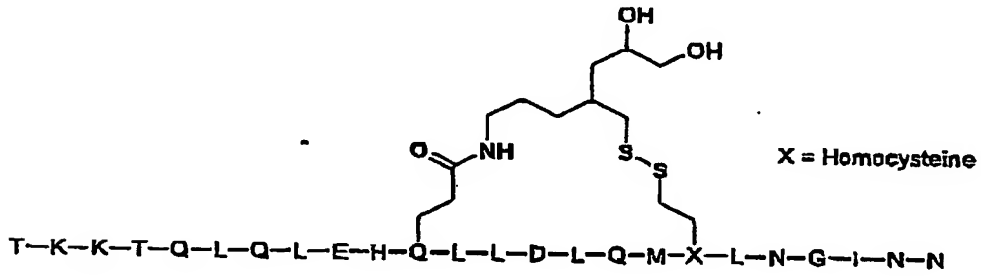
The bridge in example 3 connects the side chains of glutamine (glutamic acid respectively) and homocysteine via glycine and 2-aminoethanthiol. This compound represents an antagonist for the interleukin-2 receptor.

Example 4:



The bridge in example 4 connects the side chains of asparagine (aspartic acid respectively) and cysteine via beta-alanine and 3-aminopropan-1-thiol. This compound represents an antagonist for the interleukin-2 receptor.

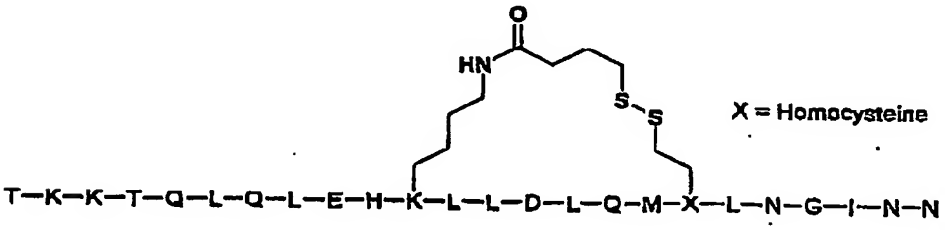
Example 5:



The bridge in example 5 connects the side chains of glutamine (glutamic acid respectively) and homocysteine via 5-aminopentan-1-thiol. The bridge backbone ist substituted with a sidechain containing two hydroxyl groups to improve the solubility of the compound.

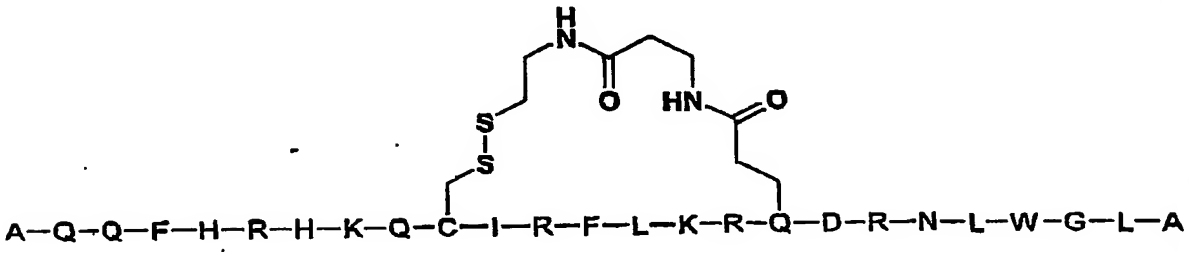
This compound represents an antagonist for the interleukin-2 receptor.

Example 6:



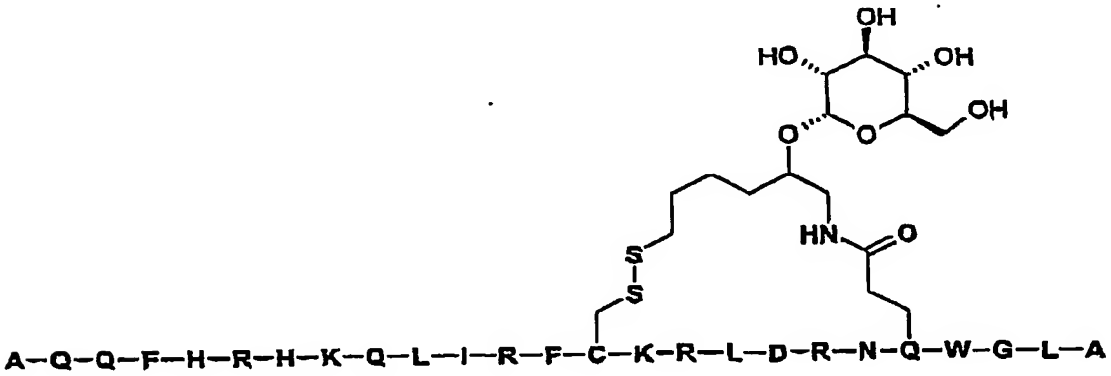
The bridge in example 6 connects the side chains of lysine and homocysteine via 3-thiopropionic acid. This compound represents an antagonist for the interleukin-2 receptor.

Example 7:



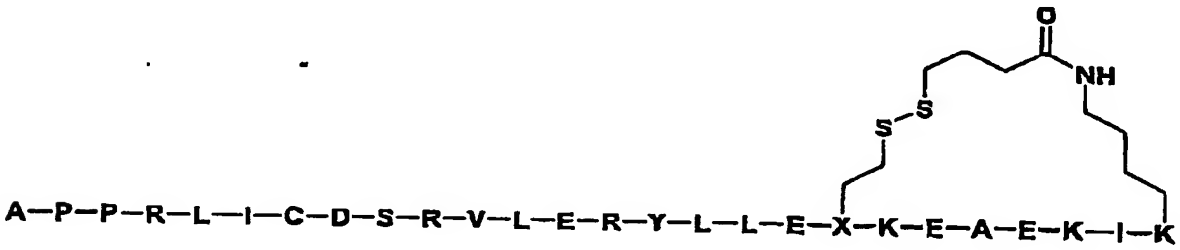
The bridge in example 7 connects the side chains of cysteine and glutamine (glutamic acid respectively) via beta-alanine and 2-aminoethanthiol. This compound represents an antagonist for the interleukin-4 receptor.

Example 8:

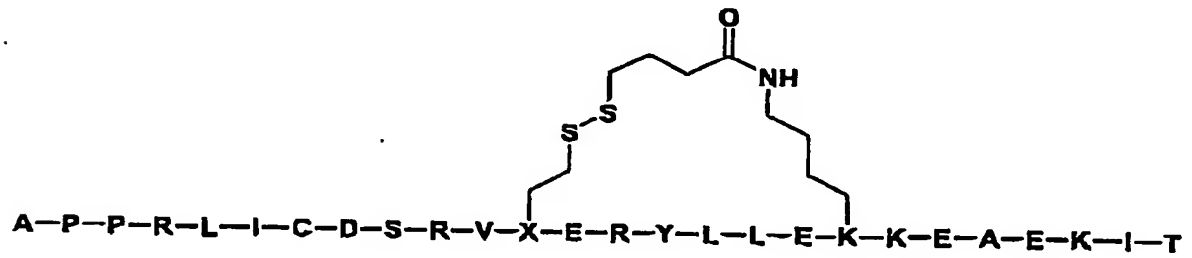


The bridge in example 8 connects the side chains of cysteine and glutamine (glutamic acid respectively) via omega-aminohexanthiol which is glycosylated to improve the pharmacokinetic properties of the compound. This compound represents an antagonist for the interleukin-4 receptor.

Example 9:



Example 10:



The bridges in example 9 and example 10 connect the side chains of homocysteine and lysine via 4-thiobutyric acid. This compounds represent binding molecules for the erythropoietin receptor.

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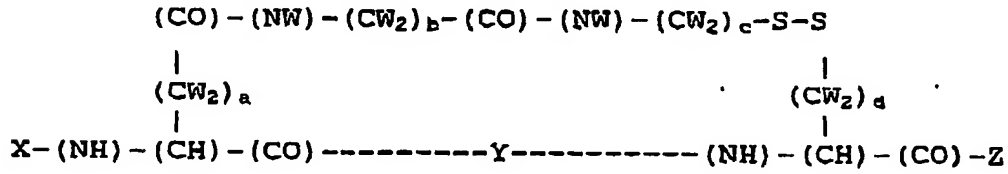
Claims

1. Peptidic compound having covalently closed bridge structures, which branch off from suitable amino acid side chains of a peptidic binding molecule with alpha-helical conformation and which connect at least two amino acid side chains of this peptide, thereby stabilizing the bridged part of the helix and being characterized by the presence of at least one amide (peptide) bond and at least one disulphide bridge, which both form part of the bridge backbone.

2. Compound according to claim 1, and represented by the molecules covered by the generic formula (1),

Formula (1):

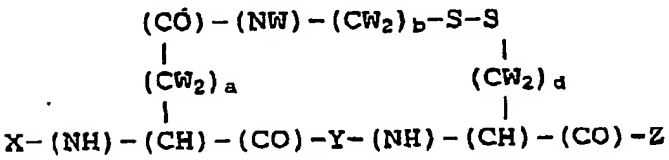


(1)

wherein X is hydrogen or any amino acid or any peptide or any compound represented by formula (1), (2) or (3), Y is any amino acid sequence consisting of six amino acids, Z is hydroxyl or any amino acid or any peptide or any compound represented by formula (1), (2) or (3); a, b, c and d are independently selected from the integers 1 to 3, provided that the sum a+b+c+d is any integer in the range from 5 to 9, at each independent

position of W, W can be freely chosen from hydrogen, a hydroxyl-, carboxyl- or amino group, an alkyl moiety with at least one hydroxyl-, carboxyl- or amino group, a polyethyleneglycol moiety, or a naturally occurring or artificial sugar molecule, and the peptides can consist of natural and/or unnatural D- and/or L-amino acids.

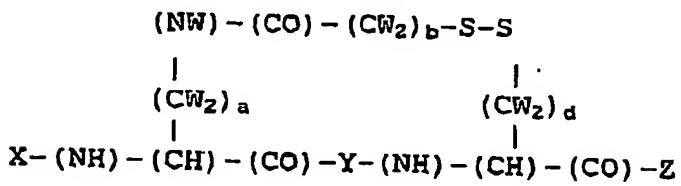
3. Peptidic compound according to claim 1, and represented by the molecules covered by the generic formula (2):



(2)

wherein X is hydrogen or any amino acid or any peptide or any compound represented by formula (1), (2) or (3), Y is any amino acid sequence consisting of six amino acids, Z is hydroxyl or any amino acid or any peptide or any compound represented by formula (1), (2) or (3), a, b and d are independently selected from the integers 1 to 5, provided that a+b+d is any integer in the range from 7 to 11; at each independent position of W, W can be freely chosen from hydrogen, a hydroxyl-, carboxyl- or amino group, an alkyl moiety with at least one hydroxyl-, carboxyl- or amino group, a polyethyleneglycol moiety, or a naturally occurring or artificial sugar molecule, and the peptides can consist of natural and/or unnatural D- and/or L-amino acids.

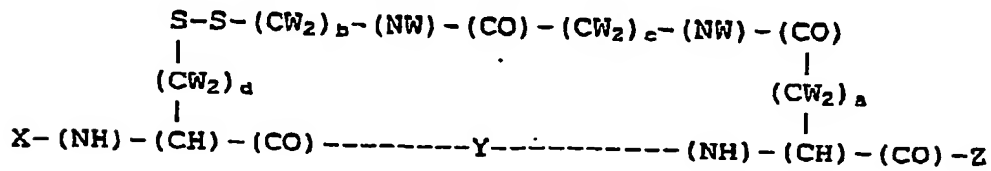
4. Peptidic compound according to claim 1, and represented by the molecules covered by the generic formula (3):



(3)

wherein X is hydrogen or any amino acid or any peptide or any compound represented by formula (1), (2) or (3), Y is any amino acid sequence consisting of six amino acids, Z is hydroxyl or any amino acid or any peptide or any compound represented by formula (1), (2) or (3), a, b and d are independently selected from the integers 1 to 5, provided that a+b+d is any integer in the range from 7 to 11, at each independent position of W, W can be freely chosen from hydrogen, a hydroxyl-, carboxyl- or amino group, an alkyl moiety with at least one hydroxyl-, carboxyl- or amino group, a polyethyleneglycol moiety, or a naturally occurring or artificial sugar molecule, and the peptides can consist of natural and/or unnatural D- and/or L-amino acids.

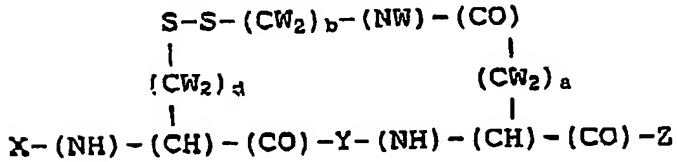
5. Peptidic compound according to claim 1, and represented by the molecules covered by the generic formula (4):



(4)

wherein X is hydrogen or any amino acid or any peptide or any compound represented by formula (1) to (2), Y is any amino acid sequence consisting of six amino acids, Z is hydroxyl or any amino acid or any peptide or any compound represented by formula (1) to (6), a, b, c and d are independently selected from the integers 1 to 3, provided that a+b+c+d is any integer in the range from 5 to 9, at each independent position of W, W can be freely chosen from hydrogen, a hydroxyl-, carboxyl- or amino group, an alkyl moiety with at least one hydroxyl-, carboxyl- or amino group, a polyethyleneglycol moiety, or a naturally occurring or artificial sugar molecule, and the peptides can consist of natural and/or unnatural D- and/or L-amino acids.

6. Peptidic compound according to claim 1, and represented by the molecules covered by the generic formula (5):

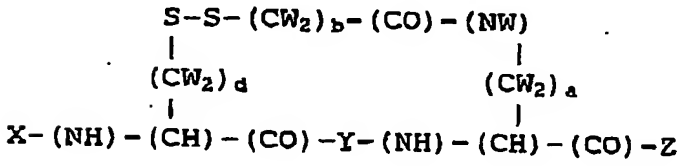


(5)

wherein X is hydrogen or any amino acid or any peptide or any compound represented by formula (1) to (6), Y is any amino acid sequence consisting of six amino acids, Z is hydroxyl or any amino acid or any peptide or any compound represented by formula (1) to (6), a, b and d are independently selected from the integers 1 to 5, provided that a+b+d is any integer in the range from 7 to 11, W is hydrogen, a hydroxyl-, carboxyl- or amino group,

an alkyl moiety with at least one hydroxyl-, carboxyl- or amino group, a peptide of maximally 30 amino acids, a polyethyleneglycol moiety, or a naturally occurring or artifical sugar molecule and the peptides can consist of natural and/or unnatural D- and/or L-amino acids.

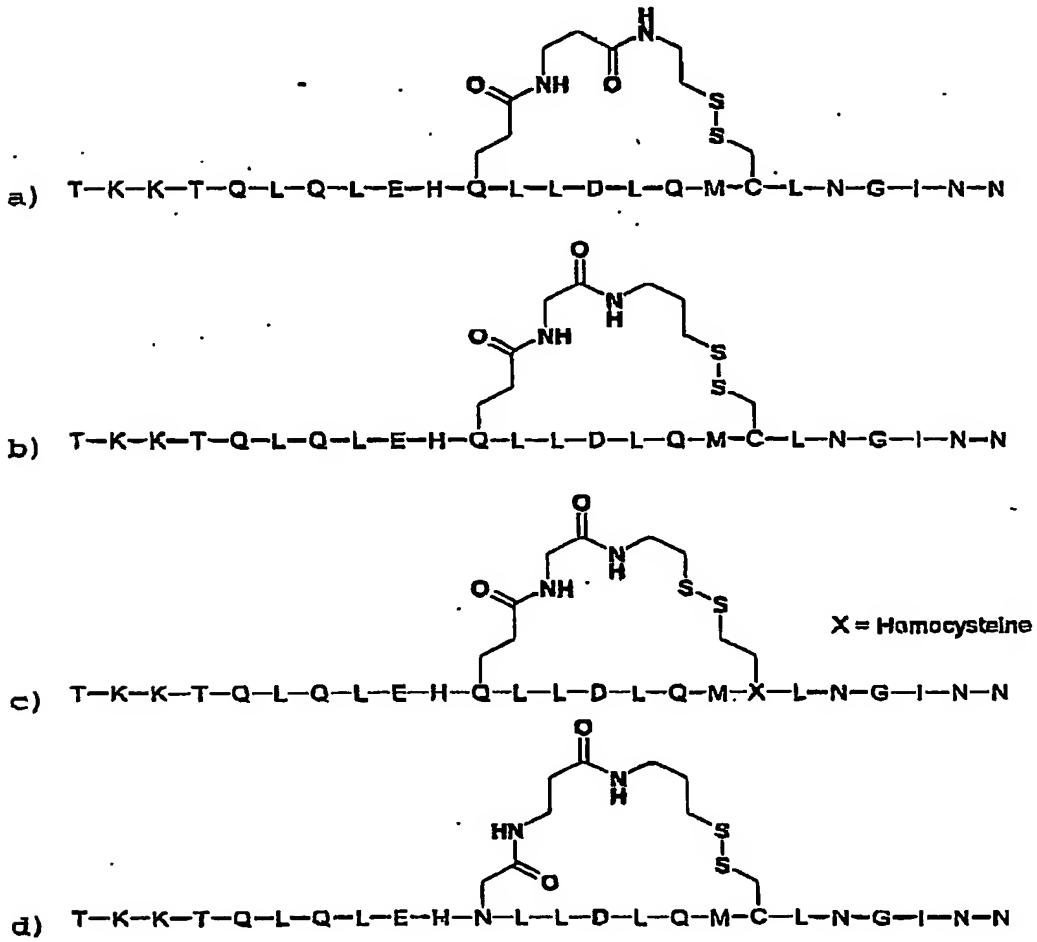
7. Peptidic compounds according to claim 1, and represented by the molecules covered by the generic formula (6):

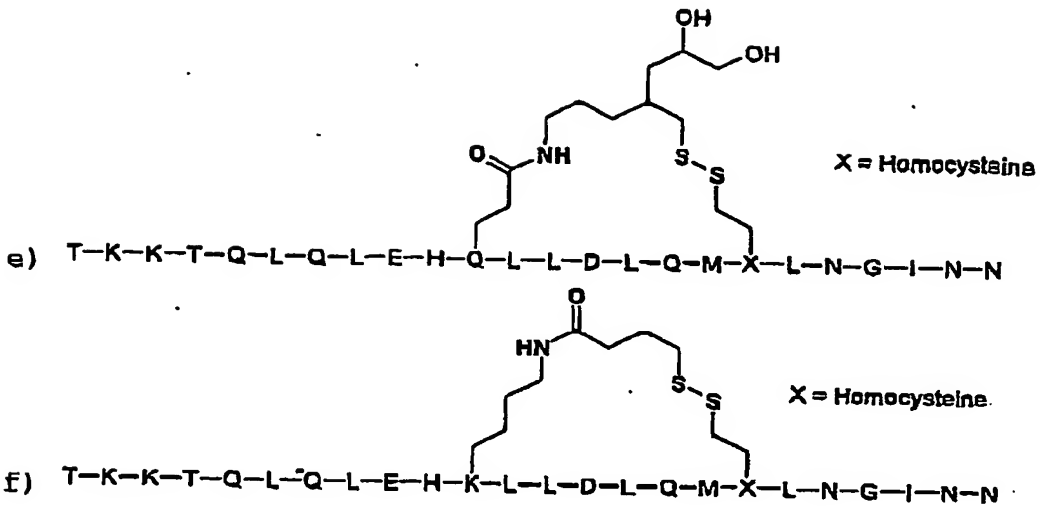


(6)

wherein X is hydrogen or any amino acid or any peptide or any compound represented by formula (1) to (6), Y is any amino acid sequence consisting of six amino acids, Z is hydroxyl or any amino acid or any peptide or any compound represented by formula (1) to (6), a, b and d are independently selected from the integers 1 to 5, provided that a+b+d is any integer in the range from 7 to 11, at each independent position of W, W can be freely chosen from hydrogen, a hydroxyl-, carboxyl- or amino group, an alkyl moiety with at least one hydroxyl-, carboxyl- or amino group, a polyethyleneglycol moiety, or a naturally occurring or artificial sugar molecule, and the peptides can consist of natural and/or unnatural D- and/or L-amino acids.

8. Peptidic compound according to claims 1- 7, binding to the interleukin 2 receptor and containing the stabilized peptide sequence TKKTQLQLEHKLLDLQMXLNGINN in a helical conformation, where X stands for homocysteine and two helical turns are bridged by a backbone according to claims 1-7; thereby including non-exclusively the sequences and structures (a- f) as follows:





9. Peptidic compound according to Claim 8, in which the bridging structure is shifted along the peptide sequence in such a way that binding to the interleukin 2 receptor is maintained and another part of the overall helical structure is bridged by the construct.

10. Peptidic compound according to claims 8 and 9, in which at least one amino acid of the peptide sequence is replaced by physicochemically related natural or non-natural amino acids in a conservative exchange, which maintains the binding of the peptide to the Interleukin 2 Receptor.

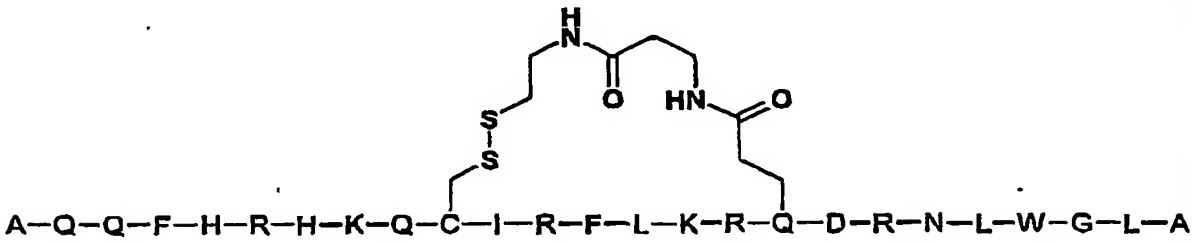
11. Peptidic compound according to claims 8 -10, which are N- and/or C-terminally modified in such a way that the binding of the peptide to the Interleukin 2 receptor is maintained and/or water solubility is improved and/or that exopeptidases can not cleave at the terminal sites, whereby

terminal modifications include non-natural amino acids, D-amino acids, sugar moieties or freely chosen appropriate organic moieties.

12. Pharmaceutical preparations containing an active ingredient according to claims 8-11 and intended for use in humans or animals as an antagonist of the action of the cytokine Interleukin 2.

13. Peptidic compound according to claims 1- 7, binding to the interleukin 4 receptor and containing the stabilised peptide sequence AQQFHRHQCIRFLKRQDRNLWGLA in a helical conformation, wherein two helical turns are bridged by a backbone according to claims 1-7; thereby including non-exclusively the following sequence and structure (g):

g)



14. Peptidic compound according to Claim 13, in which the bridging structure is shifted along the peptide sequence in such a way that binding to the interleukin 4 receptor is maintained and another part of the overall helical structure is bridged by the construct.

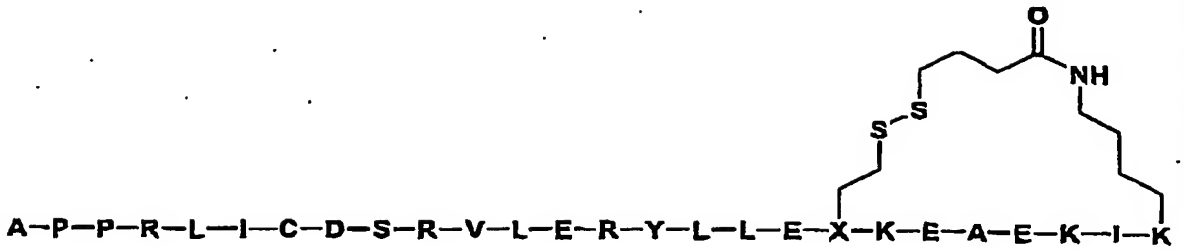
15. Peptidic compound according to claims 13-14, in which at least one amino acid of the peptide sequence is replaced by physicochemically related natural or non-natural amino acids in a conservative exchange, which maintains the binding of the peptide to the Interleukin 4 Receptor.

16. Peptidic compound according to claims 13-15, which are N- and/or C-terminally modified in such a way that the binding of the peptide to the Interleukin 4 receptor is maintained and/or water solubility is improved and/or that exopeptidases can not cleave at the terminal sites, whereby terminal modifications include non-natural amino acids, D-amino acids, sugar moieties or freely chosen appropriate organic moieties.

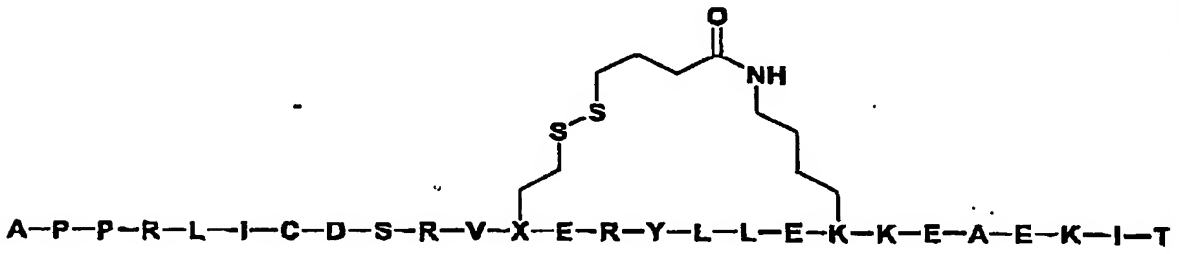
17. Pharmaceutical preparations containing an active ingredient according to claims 13-16 and intended for use in humans or animals as an antagonist of the action of the cytokine Interleukin 4.

18. Peptidic compound according to claims 1- 7, binding to the erythropoietin receptor and containing the stabilised peptide sequence APPRLICDSRVLERYLLEXKEAEKIK in a helical conformation, wherein two helical turns are bridged by a backbone according to claims 1-7; thereby including non-exclusively the following sequences and structures (h-i):

h)



i)



19. Peptidic compound according to Claim 18, in which the bridging structure is shifted along the peptide sequence in such a way that binding to the erythropoietin receptor is maintained and another part of the overall helical structure is bridged by the construct.

20. Peptidic compound according to claims 18-19, in which at least one amino acid of the peptide sequence is replaced by physicochemically related natural or non-natural amino acids in a conservative exchange, which maintains the binding of the peptide to the erythropoietin receptor.

21. Peptidic compound according to claims 18-20, which are N- and/or C-terminally modified in such a way that the binding of the peptide to the erythropoietin receptor is maintained and/or water solubility is improved and/or that exopeptidases can not cleave at the terminal sites, whereby

terminal modifications include non-natural amino acids, D-amino acids, sugar moieties or freely chosen appropriate organic moieties.

22. Pharmaceutical preparations containing an active ingredient according to claims 13-16 and intended for use in humans or animals as an agonist of the action of the cytokine erythropoietin.

23. Mono- and polyclonal antibodies to the substances covered by Claims 1-23, and the use of such antibodies in diagnostic and pharmacological quantification and/ or inhibition of action of the active substances in body fluids or tissues of animals or humans.

24. Peptidic compound according to claims 1-11, 13-16, 18-21, in which the N-terminal amino acid is acetylated and/or the C-terminal amino acid is amidated.

Abstract

The present invention therefore presents modules, from which helical constraints can be built by very flexible strategies. The peptide bonds involved partially compensate the hydrophobic nature of the disulphide bonds, which are also included into the constraint strategy. Thus, the invention presents solutions, by means of which peptide bonds or closure of disulphide bridges can be used alternatively for closure of the constraint. This offers greater synthetic flexibility. Moreover, the peptide bonds are more hydrophilic than disulphide bridges alone and offer the advantage of better solubility of the product in an aqueous surrounding. It is possible to attach solvation tags like glycosyl moieties, polyethylenglycol or other suitable extensions or appendices to the helical constraint structure. Usually, such a hydrophilic helical constraint structure replaces two hydrophobic amino acid side chains and thus improves pharmacologic properties of the molecule.